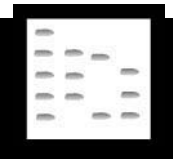
CAROTENOID PIGMENTS: SUPERCRITICAL FLUID CHROMATOGRAPHY



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Introduction

Carotenoids are one of the main classes of natural pigments and are found in a large number of fruits and vegetables (oranges, tomatoes, carrots) and in leaves where their presence is masked by chlorophylls until autumn. They are also found in some animal products (eggs, milk) and seafoods. Typically, carotenoids contain eight isoprenoid units bonded such that the units are reversed at the centre of the molecule. With this arrangement, many carotenoids are symmetrical in nature. The pigmentation of these tetraterpenes is a result of the chromophore created by the series of conjugated double bonds. The structural formula of all carotenoids is derived from that of lycopene, starting with different structural modifications which include hydrogenation, cyclization, oxidation or dehydrogenation. The oxygenated derivatives are known as xanthophylls and bare either the epoxy, carbonyl or hydroxy ester functions on their extremity or terminal ring, while the hydrocarbon carotenoids are referred to as carotenes. The structures of some basic carotenoids are given in **Figure 1**.

Carotenoids have been used for many years in the food industry as colouring material. These compounds also have many biochemical roles, including the important functions of light harvesting and photoprotection in photosynthesis. In humans, the primary use of carotenoids, whether taken as carotenoid-containing food or as dietary supplements, is the prevention or correction of vitamin A deficiency. However individual carotenoids are capable of forming different *cis*/*trans* geometrical isomers and this in turn is known to affect their biochemistry in certain situations. In fresh plant tissue, all the double bonds have the all-*trans* configuration (most stable structural form); however, isomerization to the *cis* configuration results in a loss of nutritional value. Hence the determination of these isomers is necessary for the quality control of fresh foodstuffs in order to assess the pro-vitamin A activity of foods and for the evaluation of the effects on food-processing on freshness. Feeding studies have also shown that *cis* isomers of β-carotene have lower pro-vitamin A activities when compared to the all-*trans* form. β-Carotene, besides having the highest pro-vitamin A activity of the carotenoids, has also been reported to have anti-neoplastic activity, perhaps due to their antioxidant and free radical quenching activity, not only at the stage of onset of the disease but also on existing tumours. Hence the importance of the constituents, in terms not only of colour but also nutrition, explains why attempts have been made to characterize and determine these pigments which occur together in mixtures that can be resolved only with the mildest and most selective analytical methods. While the existence of carotenes and xanthophylls was known before 1906, it was not until Tswett developed column chromatography that much was known about the carotenoids. Subsequently, chromatographic methods have improved drastically from countercurrent distribution, gas chromatography (GC), thin-layer chromatography, gravity column chromatography through to high performance liquid chromatography (HPLC).

Recently, a number of studies have been performed involving the separation of carotenoids by either supercritical (SFC) or subcritical fluid chromatography, showing that SFC with carbon dioxide as the mobile phase can provide an alternative to the traditional HPLC methods used. A brief history of the SFC of carotenoids up to the present is summarized in **Table 1**. Supercritical fluids have lower viscosities than liquids and thus solute diffusion coefficients are higher than in conventional solvents. Furthermore, the low critical temperature of some fluids enables many heat-sensitive compounds to be separated without degradation. Manipulation of various parameters such as temperature, pressure, mobile phase, modifiers and stationary-phase type makes complex separations possible. These aspects are considered in more detail in subsequent sections.

Effect of Mobile Phase and Modifiers on Carotene Separations

Supercritical CO_2 has been the most commonly used mobile phase in SFC. It is nontoxic and also has a low critical temperature (31°C), which enables the separation of thermally labile compounds such as carotenoids at low column temperatures. Furthermore, it

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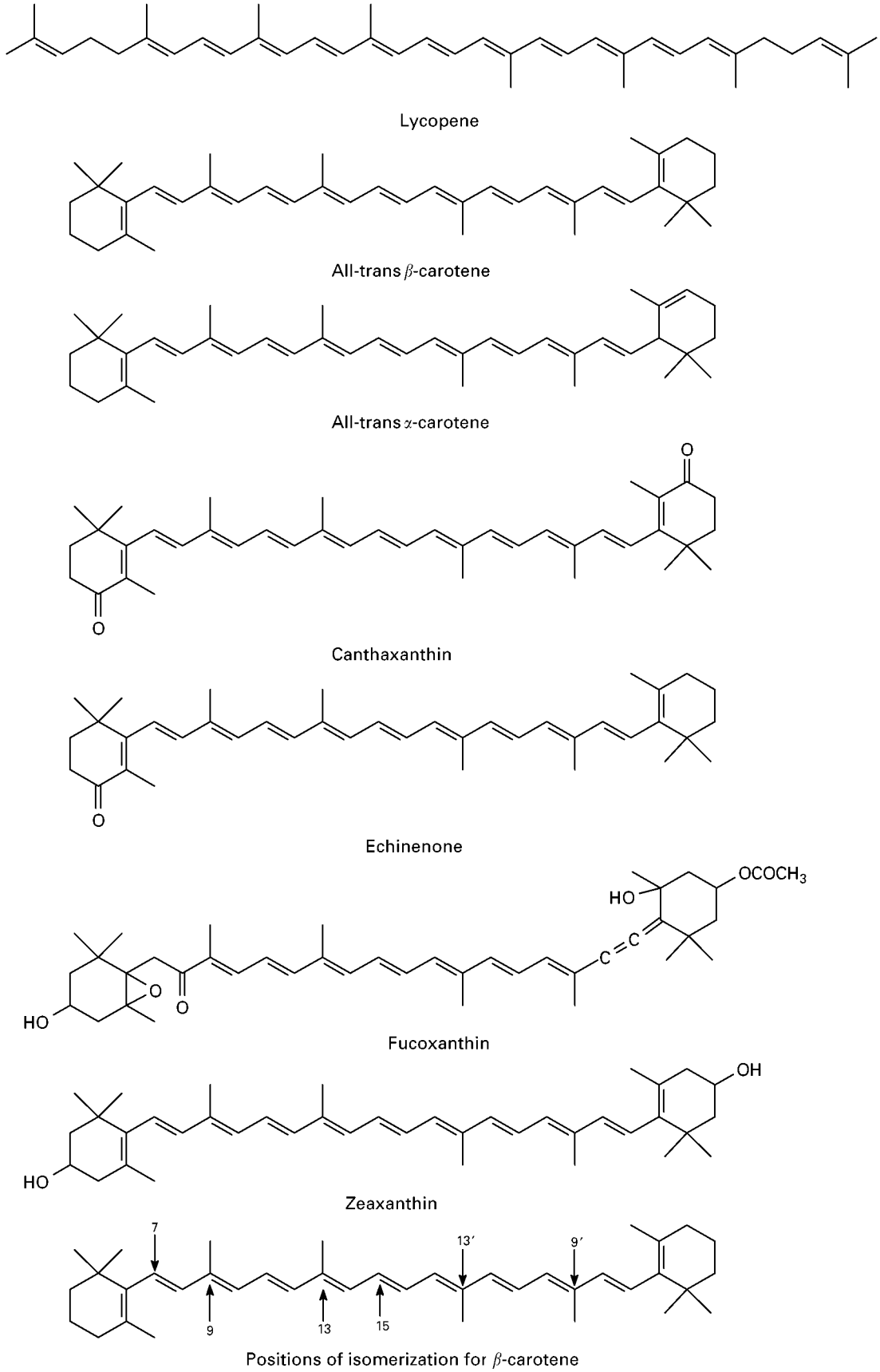

Figure 1 Chemical structures of some basic carotenoids.

Table 1 Advances in SFC of carotenoid pigments

Date	*Development*
1968	Separation of α- and β-carotene by packed-column SFC (Giddings *et al.*)
1983	Separation of lycopene and α- and β-carotene (Gere)
1989	Separation of geometrical isomers of α- and β-carotene by open tubular SFC (Schmitz *et al.*)
1991	Understanding of the effect of temperature, pressure, modifier and stationary-phase type on carotene separations (Lesellier *et al.*, Aubert *et al.*)
1994	Investigation of spectral shifts of carotenoids in supercritical CO_2 (Hui *et al.*)

is compatible with a wide range of HPLC and GC detectors. Nitrous oxide exhibits a polarity similar to CO_2 but has only received limited use because of its oxidizing properties. Nevertheless, both of these mobile phases have been investigated with β-carotene as a probe molecule. Interestingly, the retentive properties of the polar amino stationary phase for β-carotene are largely affected by the CO_2 and N_2O mobile phases, in comparison to the nonpolar octadecylsilyl stationary phase, which is not appreciably affected by these mobile phases.

In cases where increased solubilizing power is required to elute components of interest, modifiers have been successfully added to the supercritical fluid. The resulting effect is reduced solute retention, improvement of chromatographic efficiency and, in some cases, altered elution order. One of the advantages of supercritical CO_2 is that it is chemically compatible and miscible with a large number of modifiers. The effects of a range of modifiers – methanol, acetonitrile, tetrahydrofuran, dichloromethane and trichlorotrifluoroethane – on the SFC of carotenes have been studied using binary and ternary mixtures with CO_2 containing 3–20% (v/v) of the modifiers. Addition of each of these modifiers produces a concentration-dependent decrease in the capacity factors (k') of the carotenes. The effectiveness of the modifiers has not been directly correlated with their densities nor with their polarities, and this suggests that specific interactions between the solutes and the modifiers are important.

Minimal retention is obtained with the strongest modifier, dichloromethane, which is closest to the dipole–dipole interaction of Snyder's triangle. Furthermore, increasing the dichloromethane concentration in the CO_2 results in an overall decrease in retention of the carotenes and also a decrease in selectivity, probably due to reduced interactions between the solutes and the stationary phase, as all the carotenes have high affinities for this solvent. A plot of $1/k'$ against modifier concentration is linear for a mixture of tetrahydrofuran–methanol in CO_2 whereas, for more polar modifiers, the line curves slightly downwards due to increased solvent polarity, which indicates that solute–solvent interactions are important. On the other hand, an exponential curve is observed for the nonpolar modifiers, suggesting that the solubility of the carotenes is enhanced by an amount independent of the concentration of the modifier.

The selectivity between the *trans* and *cis* isomers is unaffected by the modifiers, whereas the selectivity between the α- and β-all-*trans* compounds diminishes with increasing percentage of modifier. With a CO_2–methanol–acetonitrile mixture, the selectivity between *trans* and *cis* isomers falls as the proportion of acetonitrile is increased, for both the α- and β-carotenes. This phenomenon has been used to advantage in optimizing the separation of the components of a carrot extract, as shown in **Figure 2**. With methanol–carbon dioxide, the peaks were not well resolved; however, replacing some of the methanol with acetonitrile allowed on additional component in the extract to be resolved and eluted before all-*trans* α-carotene.

Effect of Stationary Phase Type on Retention and Selectivity of Carotenoids

Both open tubular and packed columns have been employed in the separation of carotenoids. Several β-carotene isomers have been separated on an SB-Cyanopropyl-25 (25% cyanopropyl–75% methylpolysiloxane) stationary phase employing supercritical CO_2 with 1% ethanol as the mobile phase. Separation of the isomers of α-carotene however requires the use of a SB-cyanopropyl-50 (50% cyanopropyl–50% methyl-polysiloxane) stationary phase. A mixture of β-carotene, echinenone, canthaxanthin, astacene and fucoxanthin has also been separated on open tubular columns with cross-linked poly(cyanopropyl)methylsiloxane and Carbowax stationary phases. However, the more polar carotenoids did not elute.

One of the problems with immobilized polar stationary phases in open tubular columns is their instability towards higher concentrations of polar modifiers. Consequently, both polar and nonpolar packed columns have been investigated for SFC of carotenoids. The retention mechanism in normal-phase columns is based on the lattice-fluid model where the important property of the mobile phase in β-carotene retention is absorptivity as well as solubilizing power. Elution of carotenoids from normal-phase alumina and silica particles can be compared to that of normal-phase LC; however, the materials are strong

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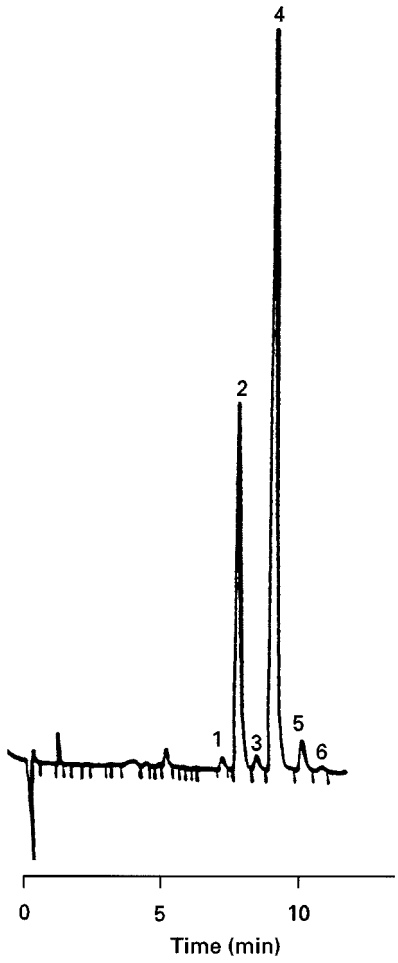

Figure 2 Separation of a carrot extract by supercritical fluid chromatography using an Ultrabase UB 225 column (250 × 4.6 mm i.d.); mobile phase, CO_2–methanol–acetonitrile (85 : 0.75 : 14.25, v/v/v); pressure, 15 MPa; temperature, 22°C; flow rate, 3.0 mL min^{-1}; detection, 450 nm. Peak identification: 1, γ or ζ-all-*trans*-carotene; 2, all-*trans*-α-carotene; 3, *cis*-α-carotenes; 4, all-*trans*-β-carotene; 5, *cis*-β-carotene; 6, *cis*-β-carotene. Reproduced with permission from Aubert *et al.* (1991).

adsorbents and can prove too adsorptive for CO_2 to elute the more polar analytes. The reversed-phase packings modified with C_8 and C_{18} exhibit dipole–dipole interactions from the surface silanols in addition to the desired dispersion characteristics and have been successfully applied to carotenoid analysis.

The retention of β-carotene on octadecylsilyl (ODS) columns is well correlated to its solubility in the mobile phase. Both the type and the percentage carbon loading of the stationary phase influence the separation of the carotenes by SFC. Among factors that determine the performance of a column, such as the shape and size of the particles, the pore size, the specific surface area and the percentage surface coverage, the kind of function that is bonded to the support is particularly important.

The effect of the stationary phase can be evaluated from overall variations in retention factors or from variations in the extent of separation of different pairs or groups of compounds, such as first, α- and β-carotenes (the isomers not being separated) and second, *cis* and *trans* isomers of β-carotene, or *cis*/*trans* α- and β-carotenes.

The relationship between the retention factor in SFC and carotene solubility in the mobile phase can be given by the following equations (as long as the same kind of fluid is used as a mobile phase):

$$\ln k^{R} = \ln \phi + \ln C_{st}^{0} + \frac{(\mu_{ss}^{0} - \mu_{st}^{0})}{RT} - \ln S \qquad [1]$$

$$S = a_{m}^{sat} C_{m}^{0} \qquad [2]$$

where ϕ is the volume ratio of the stationary and mobile phases, C_{st}^{0} is the standard (surface or volume) concentration in the stationary phase, μ_{ss}^{0} is the chemical potential of the pure solid solute at standard pressure, μ_{st}^{0} is the chemical potential of the solute in the stationary phase as it moves toward infinite dilution and standard concentration and pressure, a_{m}^{sat} is the activity of the solute at saturation in the solvent and C_{m}^{0} is the standard concentration of the solute in the mobile phase. The first, second and third terms on the right-hand side of eqn [1] are constant for a particular column, solute and temperature. If the property of the stationary phase is independent of the nature and pressure of the mobile phase, the solute capacity factor is a function of only the solute solubility in the mobile phase, regardless of the kind of solvent.

The effect of the nature of the stationary phase on carotene retention should be more significant in SFC compared with LC because the interaction between a solute and a supercritical fluid is generally small. In fact, some researchers have reported that solute retention in SFC is sensitive to the properties of the stationary phase. Where the stationary phase is prepared with a monofunctional alkylsilane, there is one-to-one bonding between the reagent and the silanol groups, giving a 'brush-type' structure. Di- and trifunctional silanes can bond to more than one silanol group on the silica support, to give essentially the same type of brush-type stationary phases as monofunctional silanes. However, they can also polymerize in the presence of traces of water. Under suitable conditions, a stationary phase can also be prepared in which each alkylsilane that is bonded to the surface of the silica gives rise to an arborescent–polymeric structure that is not brush-like and differs from column packings in which the support

Table 2 Effect of different stationary phases on the selectivity and resolution of carotene isomers

Column	L (mm)	V_0	*Porosity* ε (%)	*Type of bonding*	t_r *α-carotene* (min)	k^R *α-carotene*	*Separation of α- and β-carotenes*	*Selectivity of* trans/cis *β-carotene isomers*	*Resolution of* trans/*cis β-carotene isomers*	*Separation of α- and β-carotene* trans/cis isomers
Ultrabase UB 225	250	2.463	59	M	11.22	12.58	Yes	> 1	> 1.5	Yes
Spheri-5 ODS-5A	250	2.456	59	P(A)	11.40	12.85	Yes	> 1	> 1.5	Yes
LiChrospher 100 RP 18	250	2.150	68	D	8.35	10.57	Yes	> 1	> 1.5	Yes
LiChrospher 100 PR 18e	250	2.187	70	D	9.60	12.05	Yes	> 1	> 1.5	Yes
Nucleosil C_{18}	250	2.866	69	P(A)	7.38	6.66	Yes	> 1	> 1.5	Yes
ChromTech CT-Sil C_{18}	150	1.545	62	–	4.89	8.36	Yes	> 1	> 1.5	Yes
Supersphere 100 RP_{18}	250	2.106	67	D	8.16	8.02	Yes	> 1	> 1.5	Yes
Spherisorb ODS-2	100	1.029	62	P(A)	3.75	9.73	Yes	> 1	> 1.5	Yes
Supelcosil LC-PAH	150	1.768	71	P(A)	2.05	2.37	Yes	> 1	< 1.5	No
Erbasil C_{18}	150	1.656	66	P(A)	3.60	5.40	Yes	> 1	< 1.5	No
Suplex pKb-100	150	1.623	65	–	2.38	3.27	Yes	> 1	> 1.5	No
Ultracarb 5-ODS 20	150	1.789	72	–	10.32	16.20	Yes	> 1	> 1.5	No
Zorbax ODS	250	2.678	64	M	4.98	4.50	Yes	= 1	< 1.5	No
Ultrasphere ODS	250	2.520	61	M	2.48	1.86	No	= 1	–	No
Vydac 201 HS	150	1.760	70	M	5.43	8.13	No	= 1	–	No
Partisil 5 ODS-3	250	2.803	67	M	7.70	7.17	No	= 1	–	No
μBondapak C_{18}	300	2.908	70	–	6.29	5.42	No	= 1	–	No
Vydac 210 TP	150	1.680	67	P(A)	1.60	1.73	Yes	> 1	< 1.5	No
Perkin-Elmer HC-ODS/PAH	250	0.327	88	P(A)	1.25	2.05	Yes	> 1	< 1.5	No
Vydac 218 Tp	250	2.898	70	P(A)	3.28	2.32	Yes	> 1	> 1.5	No
Hypersil 15C_{18}	150	2.635	63	M	7.01	6.90	No	= 1	–	No
Synchropak SCD-100	150	1.874	75	M	1.68	1.59	Yes	> 1	< 1.5	No

M, monofunctional C_{18} groups; D, difunctional C_{18} groups; P(A), arborescent–polymeric C_{18} groups; – exact nature unknown.

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itself is polymeric. Arborescent–polymeric C_{18}-bonded stationary phases appear to be particularly suitable for separating closely related compounds that differ in the degree of planarity of their structures and have successfully been applied to SFC separation of *cis* and *trans* α- and β-carotenes. **Table 2** lists the different columns that have been evaluated for their separating capabilities of the different classes of carotenes. Particular attention should be paid to the estimation of the retention (R) factor (k') for the analytes because this parameter characterizes the stationary phase independently of factors such as size and porosity of the column. It has been found that on arborescent–polymeric columns, the separation of α- and β-carotenes is incomplete if the retention (R) factor for α-carotene is below 6. One also needs to be aware that, unlike a polar stationary phase, an ODS stationary phase is not appreciably affected by the nature of the mobile phase and that β-carotene, being much larger than the alkyl chains grafted on the silica surface, is less affected by the residual silanol groups on the silica surfaces.

Effect of Temperature and Pressure on Carotenoid Separations

The solubility of a substance in a supercritical fluid is the sum of two factors: the volatility of the substance (which is a function of temperature) and the solvating effect of the supercritical fluid (which is a function of fluid density). Hence, solubility is controlled experimentally by selecting appropriate temperatures and pressures which are important for controlling retention in SFC. These parameters influence the solvating power, efficiency and selectivity. At constant pressure, an increase in temperature decreases the density and consequently the solvating power. The temperature also influences the diffusion coefficients of the solutes in the supercritical fluid. With increasing temperature, diffusion coefficients increase and higher chromatographic efficiency results. However, one needs to exercise caution in this regard as carotenoids are thermally labile and will decompose at high temperatures. With pure carbon dioxide, the capacity factor (k^R) increases in proportion to the temperature

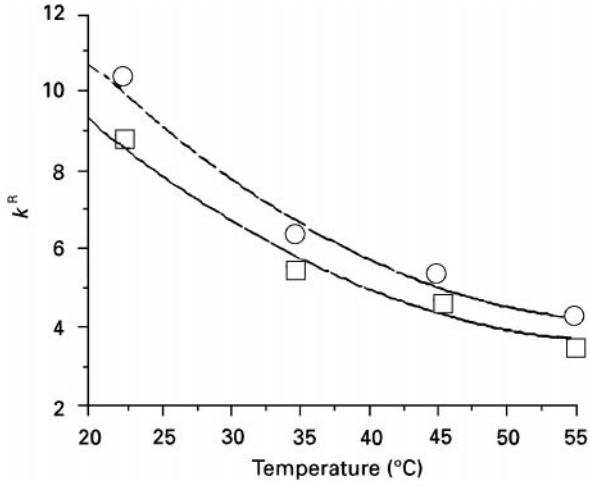

Figure 3 Dependence of k^R values of carotenes on temperature at constant pressure (25 MPa). Column, Spheri-5 ODS-5A; mobile phase, CO_2-methanol (80 : 20 v/v); flow rate, 3.0 mL min^{-1}; detection, 450 nm. Squares, all-*trans* α-carotene, circles, all-*trans* β-carotene. Reproduced with permission from Aubert *et al.* (1991).

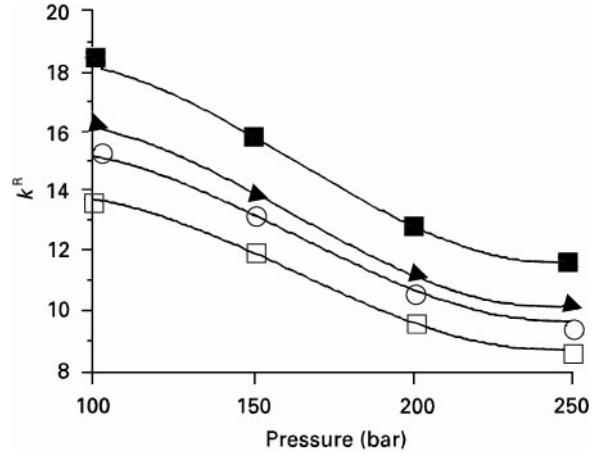

Figure 4 Dependence of k^R values of carotenes on pressure (P) at constant temperature (22°C). Column, Spheri-5 ODS-5A; mobile phase, CO_2–methanol (80 : 20 v/v); flow rate, 3.0 mL min^{-1}; detection, 450 nm. Open squares, all-*trans* α-carotene; circles, *cis* α-carotenes; triangles, all-*trans* β-carotene; filled squares, *cis* β-carotenes. Reproduced with permission from Aubert *et al.* (1991).

(at constant pressure) and can be explained by the resulting decrease in the density of the mobile phase. On the other hand, as shown in **Figure 3**, when the carotenes are eluted with carbon dioxide containing 12% methanol, k^R decreases with increasing temperature between 22 and 55°C, while the resolution between the all-*trans* α- and β-carotenes decreases as the temperature is increased. Although the optimum temperature for this separation is between 22 and 25°C, and the mobile phase is therefore subcritical, this is of little consequence because there is no discontinuity in the physical properties of the fluid at the critical point.

At constant temperature, an increase in pressure produces an increase in density. Increasing the pressure increases the mobile-phase viscosity, thus decreasing the mass transfer term C and the diffusion coefficient. In SFC, density (or pressure programming) is the primary method for developing separation. It is analogous to temperature programming in GC, and eluent composition programming in LC. Increasing the pressure at constant temperature leads to decreased capacity factors, which can be explained by the enhanced solubility of the solutes with increasing density of the mobile phase. **Figure 4** illustrates the dependence of k^R values of carotenes on pressure at constant temperature. In this work, the pressure was varied between 100 and 250 bar, at 22°C and the capacity factors were observed to decrease less rapidly at pressures above 200 bar. This is probably due to the nonlinearity of the P–T curve of carbon dioxide, which at 22°C becomes less compressible. In practice, it is preferable to avoid working at pressures where the fluid is very compressible, as the pressure drop along the column is associated with a density gradient and reduced efficiency.

Detectors in SFC with Reference to Carotenoid Analysis

SFC is compatible with a wide range of detection methods. The two basic types of detectors are the ionization detectors and optical detectors. Most commercial open tubular SFC systems provide a flame ionization detector (FID) as standard and it is therefore the most widely used detector for carotenoids. However, detection of carotenoids in supercritical fluids following chromatographic separation has also been achieved using mass spectrometry (MS) and UV/Vis detectors. SFC-MS provides detailed information on the molecular structure of eluted carotenoids and greatly aids peak identifications. Due to the thermal instability of these compounds, 'soft' chemical ionization is recommended. Use of methane as the chemical ionization reagent gas and a low source temperature of 100–120°C results in minimal fragmentation, even for thermally labile carotenoids. For more routine analysis, online UV-Vis monitoring (especially using photodiode array detection) is now the standard procedure for the analysis of carotenoids by HPLC and is the preferred detection system for the SFC analysis of carotenoids, with a number of high pressure, temperature-regulated flow cells being available. The effects of pressure and temperature on the absorption spectra of carotenoids dissolved in supercritical carbon dioxide are discussed in the following section.

Behaviour of Electronic Absorption Spectra in Supercritical CO_2

When using UV-Vis detection for SFC of carotenoids, attention must be paid to spectral shifts that occur in supercritical fluids, in comparison to those measured in liquid solvents. This is particularly relevant as the effect can influence qualitative and quantitative results. The shape of the absorption spectra of all-*trans*-β-carotene, 15-*cis*-β-carotene and the xanthophylls, zeaxanthin, cathaxanthin and astaxanthin, which are nonacidic oxygen derivatives of the carotenes, are similar in both supercritical CO_2 and hexane. The λ_{max} of the five carotenoids however shifts to a shorter wavelength (hypsochromic shift) in supercritical CO_2. **Figure 5** shows the absorption spectra of all-*trans*-β-carotene in supercritical CO_2 and in hexane. **Table 3** lists the maximum absorbance wavelength of the selected carotenoids in supercritical CO_2 and in hexane. Due to the relatively low solubilities of the xanthophylls in supercritical CO_2 below 5000 psi pressure, comparison of the spectra has been carried out at 6000 psi and 35°C. While solvent-induced shifts of the visible ($^1Bu^+$) spectra of carotenoids in a range of polar and nonpolar organic solvents commonly used for HPLC are well established, changes in the supercritical CO_2 density have also been observed to shift the λ_{max}. Density is related to both pressure and temperature, hence a change in either of the two variables is known to affect the absorption maxima of carotenoids. An increase in pressure increases the visible λ_{max} of all-*trans*-β-carotene. Over the pressure range of 1500–6000 psi (at a constant temperature of 35°C), the position of this λ_{max} shifts 7.0 nm to longer wavelength (430.0 to 437.0 nm) in a nonlinear manner.

The effect of variation in temperature on λ_{max} has also been assessed in the range 25–50°C at a constant pressure of 3000 psi and over this range only a 2.0 nm shift is observed. Thus, changes to both pressure and temperature can induce shifts in the ($^1Bu^+$) λ_{max} of all-*trans*-β-carotene in supercritical CO_2, although the former is much more significant. In addition to the main absorption peak in the visible region, *cis*-isomers of carotenoids exhibit a peak in the UV region, termed the *cis*-peak, which originates from the $^1Ag^+$ transition. The electronic absorption spectrum of 15-*cis*-β-carotene in supercritical CO_2 shows a clear *cis*-peak in the region 250–350 nm. Unlike the behaviour of the main λ_{max} in the visible region, the position of the *cis*-peak maxima does not alter as a function of temperature and pressure, but remains fixed, suggesting that the energies of the $^1Bu^+$ and $^1Ag^+$ states of carotenoids may not respond in the same manner to alternations in density.

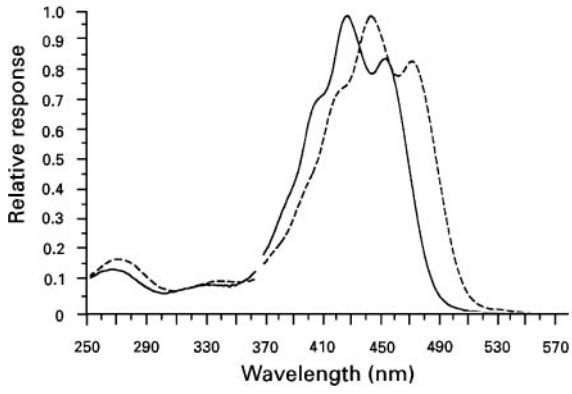

Figure 5 Absorption spectra of all-*trans*-β-carotene in supercritical CO_2 (continuous line) and in hexane (dashed line). Supercritical conditions: mobile-phase CO_2, temperature 35°C, pressure 3000 psi. Reproduced with permission from Hui *et al.* (1994).

Table 3 The maximum absorbance wavelength of carotenoids in hexane and in supercritical CO_2 (6000 psi at 35°C)

Carotenoid	λ_{max} (nm)	
	Hexane	*CO_2*
All-*trans*-β-carotene	450.0	437.0
Lycopene	472.0	456.0
Astaxanthin	474.0	453.0
Canthaxanthin	464.0	452.0
Zeaxanthin	448.0	436.0

Future Developments

As SFC continues to evolve, it will probably be increasingly applied to carotenoid analysis, particularly in view of the thermal instability of these compounds. Development of more selective stationary phases and application of micro-packed columns for SFC are key areas where improvements are likely. Micro-packed columns with internal diameters of 200–400 µm offer increased efficiencies, shorter run times and increased ease of interfacing with ionization detectors. Further, mobile phases such as the fluorocarbons have shown potential in similar applications and are therefore likely to be investigated for SFC of carotenoids.

Further Reading

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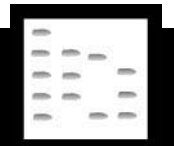
CATALYST STUDIES: CHROMATOGRAPHY



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Introduction

Catalysis is a branch of chemical kinetics of great industrial and commercial importance. Heterogeneous catalysts are certain particulate solids of high surface area ($1–300\ m^2\ g^{-1}$) that increase the rates of attaining equilibria. This is achieved by the temporary attachment of reactant molecules by moderate chemical bonds to active sites on their surfaces. Catalysts themselves are not consumed during the process, although their activity is eventually lost by surface degradation. Over 90% of the world's manufactured chemicals involve catalysis at one or more stages. The market is about $£7 \times 10^9$ per annum, with £200 return on each pound spent on a catalyst.

Background to Heterogeneous Catalysis and Contributions from Gas Chromatography

The invention of gas chromatography (GC) in 1952 and its development since then have been widely reviewed. Before looking at the applications of GC to catalysis, it is necessary to summarize the major stages in the concepts of catalysis and the often independent technological achievements of industrial scientists and engineers. **Tables 1** and **2**, respectively, contain the important concepts and terms and the major technical advances in catalysis.

Catalysis has a much longer history than GC – indeed life itself may originate from the catalysed conversion of the primitive atmosphere into bioorganic molecules on the surfaces of clay minerals. The ancients used enzymatic catalysts unknowingly in fermentation to make wine and vinegar. It is salutary that these natural catalysts far outperform any of our synthetic catalysts at ambient pressures and temperatures. Silver, regarded for at least three millennia as having preservative and curative powers and long used for storing wines and wound treatments, has now re-emerged in the sterile surface coating, Amenitrop, whose active antibacterial and antiviral ingredients are silver thiosulfate complexes.

In catalysis empirical technology and industrial innovation have almost always outpaced scientific theory and understanding, but this is less so today; exceptions include the Haber process for ammonia production. Nevertheless, it has always been true that basic studies provide the intellectual framework and accelerate technical developments, i.e. they 'catalyse' progress!

The scientific study of catalysis started soon after the beginnings of chemistry and at the time when phlogiston theory still held some sway. In 1835 Berzelius coined the term 'catalysis' (which is Greek for *to loosen*) after realizing that research findings in the first decades of the nineteenth century demonstrated